J. Trace Elem. Med. Biol. Vol. 17 (1) 51–55 (2003)
http://www.urbanfischer.de/journals/jtraceelm

Journal of
Trace Elements
in Medicine and Biology

Toxicology

Cadmium exposure in tobacco workers: possible renal effects

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Received June 2001 · Accepted August 2002

Abstract

Cadmium is a nephrotoxic metal widely used in industry and the main source of Cd in general population is smoking. Considering that the source of Cd in cigarettes is the tobacco leaf, the exposure to Cd was evaluated in workers employed at a tobacco leaf processing factory. Blood and urinary Cd levels were measured by flameless atomic absorption spectrometry in 87 workers and 35 controls. Urinary enzymes, total protein, albumin and uric acid were also determined to investigate the possible nephrotoxic effects of Cd.

Blood Cd levels were significantly higher in workers (1.63 ± 1.95 µg/L) than in controls (0.91 ± 1.15 µg/L) ($p = 0.044$). The increase observed in urinary Cd levels of workers was non significant (0.56 ± 0.5 µg/g creatinine in workers and 0.46 ± 0.5 µg/g creatinine in controls).

Both in workers and in controls, subjects smoking >10 cigarettes/day showed significantly increased blood Cd levels compared to non-smokers ($p = 0.000$ and $p = 0.011$, respectively).

In workers, urinary alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total protein, and uric acid were observed to be significantly increased ($p = 0.013$, $p = 0.000$, $p = 0.000$, $p = 0.025$, respectively), ALP, GGT and total protein being positively correlated with Cd in urine.

In conclusion, the workers in the tobacco leaf processing factory were found to be exposed to Cd compared to the general population. The increase in the urinary enzymes and proteins suggests that an exposure to Cd affects kidney functions even below the toxic limits generally accepted.

Key words: cadmium, tobacco, nephrotoxic

Introduction

Cadmium, a very cumulative element with a biological half-life of >10 years in man (1), is widely used in the industry for metal plating and for the production of batteries, pigments, plastic stabilisers, and some alloys (2, 3). In occupational setting, inhalation of Cd containing dust and fumes is the major route of Cd uptake. For the general population, the two main sources are diet (from contaminated water and crops grown on contaminated soil) and tobacco smoking. The oral absorption rate is approximately 2–7%, with values of up to 20% for subjects with very low iron stores. On the other hand, a single cigarette is determined to have 1–2 µg of Cd and 10% of inhaled Cd is absorbed by the lungs (4, 5, 6, 7).

The source of Cd in cigarettes is the tobacco leaf grown on polluted soil. In a study carried out in Turkey, the Cd content of tobacco leafs grown in the Aegean region was estimated to be 0.800 ± 0.125 µg/g (8). Considering that workers processing tobacco leafs may be exposed to Cd by inhalation of tobacco dust and/or by oral administration from contaminated hands and food in the environment, this study was undertaken to evaluate whether there is a significant Cd exposure in the workers of a tobacco factory in this region.

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0946-672X/03/17/01-051 $ 15.00/0

Cd exposure is known to have adverse effects on the kidney, liver, bones and cardio-pulmonary system, but the kidney is accepted to be the critical organ. Chronic exposure to Cd often causes renal dysfunction (2, 4, 9). The first detectable nephrotoxic effect of Cd is suggested to be an increased excretion of low molecular weight proteins and N-acetyl-β-D-glucosaminidase (NAG), an enzyme proposed as indicator of renal proximal tubular damage (2, 10, 11, 12, 13). The excretion of other enzymes – Alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT) –, glucose, amino acids, and uric acid may also reflect the renal tubular dysfunction (1, 2, 12, 14). In this study, the possible nephrotoxic effects of Cd have been evaluated in the workers of the tobacco processing factory.

Materials and methods

Eighty seven workers who had been working for 5 to 28 years (mean ± SD: 13.3 ± 7.6 years) in a tobacco leaf processing factory in Izmir, in the Aegean region, were examined. The control group consisted of thirty five age and sex matched subjects with no history of exposure to Cd. The workers were aged between 26 and 50 years (35.6 ± 7.8), the controls between 13 and 58 years (38.3 ± 11.5).

Blood samples were obtained by venipuncture using Cd free disposable syringes. An early morning urine sample was collected and adjusted to pH 7. Samples were collected and stored in Cd free tubes.

The Cd content of blood and urine was determined by graphite furnace atomic absorption spectrometry (AAS-680, Shimadzu Co, Japan), using graphite tubes with platform (Shimadzu Co, Japan). The method was modified from D'Haese et al. and a matrix matched calibration was performed (15).

Matrix modifier for blood Cd: 2.5 g diammonium hydrogen phosphate [$(NH_4)_2HPO_4$] was dissolved in 1 mL HNO_3. After addition of 1 mL Triton X 100, the volume was filled up to 500 mL with type I water.

Matrix modifier for urinary Cd: 1.5 g $(NH_4)_2HPO_4$ was dissolved in 5 mL HNO_3 and the volume was filled up to 500 mL with type I water.

Blood and urine samples obtained from healthy subjects with no history of exposure to Cd were pooled for preparing matrix matched working standards.

The stock standard of 1000 mg/L was prepared in type I water. Intermediate standards of 2, 4, 8, and 16 µg/L were prepared in 2 mL/L HNO_3. For working standards of 0.4, 0.8, 1.6, and 3.2 µg/L, 1 mL of each corresponding intermediate standard (2, 4, 8, 16 µg/L respectively) was added to 1 mL of pool (blood or urine) and 3 mL of matrix modifier.

For sample preparation 100 µL of sample was added to 400 µL of matrix modifier.

Analytical conditions for the measurement of blood and urine Cd are summarized in Table 1. For the blood Cd analysis, within run CV was 8.6% at 4.03 µg/L (n = 10) and between run CV was 14.0% at 7.64 µg/L (n = 10); for urinary Cd analysis these values were 8.9% at 1.97 µg/L (n = 14) and 13.6% at 2.41 µg/L (n = 10) respectively. The recovery rates for blood and urinary Cd were 92% at 0.80 µg/L and 103% at 0.80 µg/L respectively. Detection limits were determined to be 0.100 µg/L for blood Cd and 0.125 µg/L for urinary Cd.

Table 1. Analytical conditions for blood and urinary Cd

General conditions	
Wavelength (nm)	228.8
Slit (nm)	0.5
Lamp current (mA)	8
Background correction	D_2-BGC
Graphite tube	Platform (pyrolitic)*
Sample volume	10 µL
Peak	Peak area (height)*

Graphite furnace conditions

Stage	Temperature (°C)	Time	Ramp/ step	Gas (mL/min)
1	120	20	ramp	500
2	250	10	ramp	500
3	800 (300)*	30 (15)*	ramp	500
4	800 (300)*	20 (10)*	step	500
5	1300	3	step	0
6	2500	3	step	500

*Values in brackets are for urinary Cd analysis.

Urinary uric acid, LDH, ALP, and GGT were determined using Randox commercial kits with a Hitachi 747–200 autoanalyser. The NAG activity in urine was determined spectrophotometrically using a Boehringer Mannheim commercial kit. The urinary protein was measured by a Randox commercial kit with a Bayer opeRA autoanalyser. Albumin in urine was determined by a nephelometric method using a Beckman array protein system autoanalyser. All urinary measurements were related to the urinary creatinine and expressed as per gram creatinine.

For statistical analysis, an unpaired, 2-tailed student's t-test, one way of variance (ANOVA) and Pearson's correlation tests were performed.

Results

Blood Cd levels in workers and controls were determined to be 1.63 ± 1.95 µg/L and 0.91 ± 1.15 µg/L respectively, the difference being statistically significant ($p = 0.0044$).

There was no significant difference between urinary Cd in workers and in the control group (workers: 0.56 ± 0.55 µg/g creatinine; control group: 0.46 ± 0.35 µg/g creatinine).

To evaluate workers and controls with respect to smoking, each group was divided into three subgroups. In workers, blood Cd levels of subjects smoking >10 cigarettes/day were higher than of both the subjects smoking <10 cigarettes/day and the non-smoking subjects. Similarly, in the control group, blood Cd levels of subjects smoking >10 cigarettes/day were higher than of the other two groups. However, in both the workers and the controls the difference was significant only between those smoking

Table 2. Comparing workers and controls with respect to different parameters in urine

	Workers (n = 87) Mean ± SD	Controls (n = 35) Mean ± SD	P value
NAG [U/g creatinine]	4.96 ± 4.42	4.42 ± 3.07	0.387
GGT [U/g creatinine]	40.1 ± 19.9	21.7 ± 12.7	**0.000**
LDH [U/g creatinine]	18.2 ± 25.4	13.6 ± 5.1	0.345
ALP [U/g creatinine]	11.7 ± 14.3	7.4 ± 3.4	**0.013**
Protein [mg/g creatinine]	91.8 ± 92.0	35.7 ± 28.4	**0.000**
Albumin [mg/g creatinine]	1.20 ± 3.36	0.92 ± 1.27	0.638
Uric acide [mg/g creatinine]	330 ± 167	271 ± 105	**0.025**

>10 cigarettes/day and the non-smokers (p = 0.000 and p = 0.011, respectively, Fig. 1).

Considering the effect of smoking on the urinary Cd levels, no significant differences could be detected between non-smokers, those smoking >10 cigarettes/day and those smoking <10 cigarettes/day neither in the worker nor in the control group (Fig. 2).

Blood and urinary Cd levels of each subgroup of workers were higher than the corresponding subgroup of controls, the difference being significant only between blood Cd levels of non-smokers in the workers (0.78 ± 0.59 µg/L) and in the control group (0.43 ± 0.41 µg/L, p = 0.009).

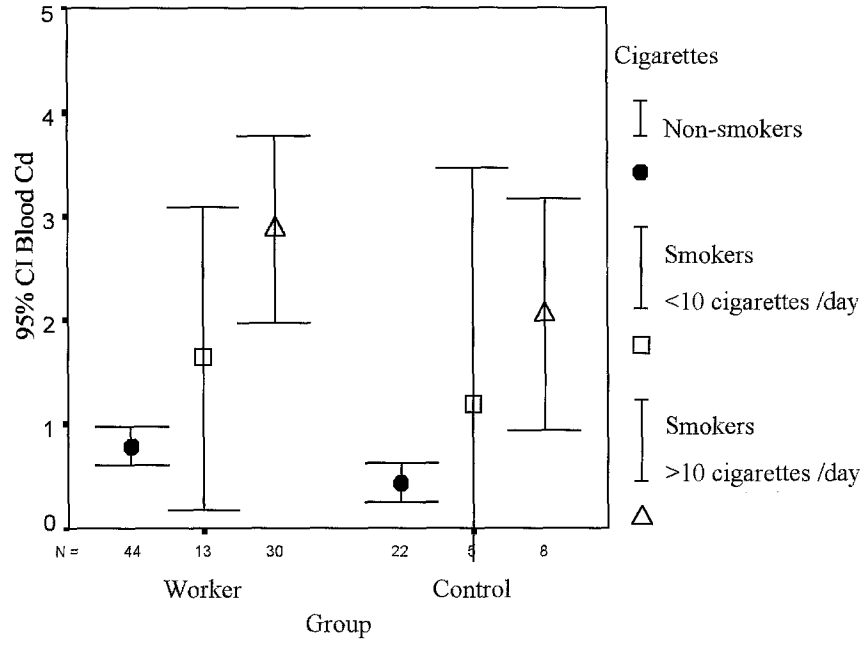

Fig. 1. Blood Cd levels in workers and controls with respect to smoking.

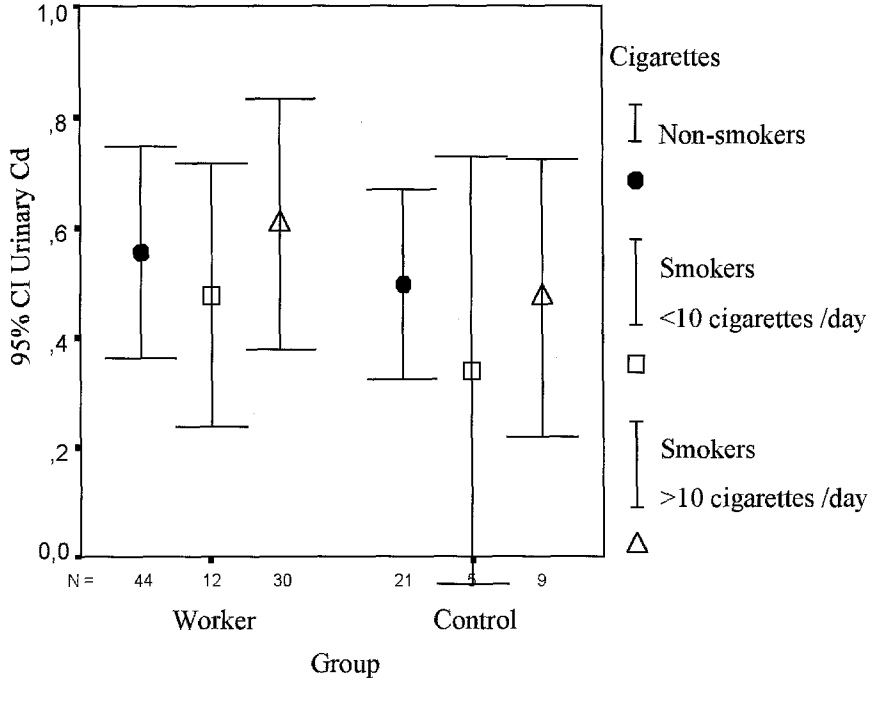

Fig. 2. Urinary Cd levels in workers and controls with respect to smoking.

The levels of the urinary enzymes NAG, ALP, LDH and GGT were higher in workers than in the control group, differences being significant for ALP and GGT ($p = 0.013$, $p = 0.000$, respectively, Table 2).

Urinary total protein, albumin and uric acid levels in workers were higher than in the controls, differences for total protein and uric acid being significant ($p = 0.000$, $p = 0.025$, respectively, Table 2).

Significant correlations between urinary Cd and GGT, ALP, and total protein were determined in workers ($r = 0.5100$, $p = 0.000$; $r = 0.2981$, $p = 0.007$; $r = 0.3071$, $p = 0.006$, respectively).

Discussion

Workers in the tobacco processing factory were determined to have significantly increased blood Cd levels in comparison to the unexposed controls. None of the workers had blood Cd levels >10 µg/L which is the limit of toxicity, while 5 of them had values >5 µg/L, the limit of exposure in the general population (1, 2, 9). In the control group, there was no subject with a blood Cd level >5 µg/L.

Previous studies have shown the increase of Cd in workers of the metal and battery industry. Although the workers of the tobacco leaf processing factory in our study did not have blood Cd levels as high as those reported for workers in the Cd-Ni or metal plating industry (2, 3, 13, 17), the significant increase with respect to the control group shows that the exposure to Cd of these workers is higher than that of the general population. It should be noted that the blood Cd levels of our controls are comparable to the values reported for the non exposed control groups of the studies mentioned above (12, 13, 17).

It is well known that cigarette smoking increases blood Cd levels and the total Cd body burden (2, 4, 18, 19). In order to rule out the effect of cigarette smoking, non-smoking workers were compared to the non-smoking controls and blood Cd levels were again determined to be significantly elevated in the worker group. This finding further supports the exposure of workers in the tobacco processing factory.

Urinary Cd is believed to reflect the amount of Cd stored in the body, particularly in the kidney. If the Cd level in urine reaches up to 10 µg/g creatinine, Cd in the renal cortex is considered to reach the critical level (200 µg/g) (2, 4, 10, 11). In this study, even though the urinary Cd levels were higher in the worker group, the difference was nonsignificant. In only two workers, urinary Cd exceeded 2 µg/g creatinine, the limit for Cd exposure (14, 23). None of the workers had urinary Cd above 10 µg/g creatinine which is considered to be the limit for toxicity.

Many studies have described evidence of tubular damage or dysfunction among workers exposed to Cd (1, 14, 20). The proximal tubular dysfunction due to the possible nephrotoxic effect of Cd was evaluated by measuring the urinary enzymes NAG, ALP, LDH and GGT, which were all elevated in workers. ALP and GGT showed a significant increase and were also positively correlated with the urinary Cd excretion.

Although the nephrotoxic effect of Cd usually gives rise to proximal tubular dysfunction, there are studies pointing out the glomerular and distal tubular dysfunction in Cd nephrotoxicity (5, 43, 47). In workers occupationally exposed to Cd, mixed proteinuria has been detected due to the excretion of high molecular weight along with low molecular weight proteins (14, 20, 21, 22). It has been stressed that, a slightly increased glomerular permeability to high molecular weight proteins may precede the occurrence of enzymuria. Furthermore, only high molecular weight proteinuria may be observed (3). In this study, urinary albumin and total protein were found to be higher in the workers. The urinary total protein was also significantly correlated with the Cd levels in urine, a finding which further supports the presence of glomerular dysfunction due to Cd exposure.

In conclusion, workers in a tobacco leaf processing factory were determined to be exposed to Cd compared to the general population. Considering the elevation in the urinary enzymes and proteins in these workers and that some of these parameters are positively correlated to urinary Cd, it may be stated that exposure to Cd affects the kidney functions even below the toxic limits generally accepted.

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